Apical extrusion of intracanal bacteria following use of two engine-driven instrumentation techniques

K. Er[1], Z. Sümer[2] & K. E. Akpınar[1]

[1]Department of Endodontics, School of Dentistry; and [2]Department of Microbiology, School of Medicine, Cumhuriyet University, Sivas, Turkey

Abstract

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Aim To evaluate the number of bacteria extruded apically from extracted teeth *ex vivo* after canal instrumentation using the two engine-driven techniques utilizing nickel-titanium instruments (ProTaper and System GT).

Methodology Forty extracted single-rooted human mandibular premolar teeth were used. Access cavities were prepared and root canals were then contaminated with a suspension of *Enterococcus faecalis* and dried. The contaminated roots were divided into two experimental groups of 15 teeth each and one control group of 10 teeth. Group 1, ProTaper group: the root canals were instrumented using ProTaper instruments. Group 2, System GT group: the root canals were instrumented using System GT instruments. Group 3, control group: no instrumentation was attempted. Bacteria extruded from the apical foramen during instrumentation were collected into vials. The microbiological samples from the vials were incubated in culture media for 24 h. Colonies of bacteria were counted and the results were given as number of colony-forming units. The data obtained were analysed using the Kruskal–Wallis one-way analysis of variance and Mann–Whitney *U*-tests, with $\alpha = 0.05$ as the level for statistical significance.

Results There was no significant difference as to the number of extruded bacteria between the ProTaper and System GT engine-driven systems ($P > 0.05$).

Conclusions Both engine-driven nickel-titanium systems extruded bacteria through the apical foramen.

Keywords: apical extrusion, bacteria, engine-driven techniques.

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Introduction

A major objective in root canal treatment is to clean the root canal system. During the process dentine chips, pulp tissue fragments, necrotic tissue, microorganisms and intracanal irrigants may be extruded through the apical foramen. This is of concern as material extruded from the apical foramen may be related to post-instrumentation pain or to a 'flare-up' (Seltzer & Naidorf 1985).

Correspondence: Dr Kürşat Er, Department of Endodontics, School of Dentistry, Cumhuriyet University, 58140 Sivas, Turkey (Tel.: +90 346 2191010; fax: +90 346 2191237; e-mail: kursater@cumhuriyet.edu.tr).

In asymptomatic chronic periradicular lesions associated with infected canals, there is a balance between microbial aggression from the infecting canal microbiota and the host defences in the periradicular tissues. During chemomechanical preparation, if the bacteria are extruded apically, the host will be faced with a challenge from a larger number of irritants than initially. Consequently, there will be a transient disruption in the balance between aggression and defence in such a way that the host will mobilize an acute inflammatory response to re-establish the equilibrium (Siqueira 2003).

Extruding bacteria and their products into the periradicular tissues can generate an acute inflammatory response, the intensity of which will depend on the

number (quantiative factor) and/or virulence (microbial species, qualitative factor) of the bacteria. However, instrumentation techniques have been demonstrated to promote apical extrusion of debris (Al-Omari & Dummer 1995), with the result that the quantiative factor is more likely to be under control of the dentist. On the contrary, the qualitative factor is more difficult to control. When virulent clonal types of pathogenic bacterial species are present in the root canal system and are propelled to the periradicular tissues during instrumentation, even a small amount of infected debris will have the potential to cause or exacerbate periradicular inflammation (Siqueira 2003). Therefore, it is logical to assume that minimizing the amount of apically extruded debris should minimize postoperative reactions.

All preparation techniques and instruments have been reported to be associated with extrusion of infected debris, even when preparation is maintained short of the apical terminus (Al-Omari & Dummer 1995, Beeson *et al.* 1998, Hinrichs *et al.* 1998, Reddy & Hicks 1998, Ferraz *et al.* 2001, Azar & Ebrahimi 2005, Tinaz *et al.* 2005). Al-Omari & Dummer (1995) verified that techniques involving a linear filing motion, such as the stepback techniques, create a greater mass of debris than those involving some sort of rotational action. Reddy & Hicks (1998) were the first to compare apical debris extrusion between hand instrumentation and engine-driven techniques. When comparing the mean weights of apically extruded debris, they noted that the stepback technique produced significantly more debris than the engine-driven technique and the balanced-force technique. Reddy & Hicks (1998) suggested that rotation during instrumentation, in both the engine-driven technique and the balanced-force technique, tended to pack the dentinal debris into the flutes of the instruments and directed them towards the orifice.

During the last decade, root canal preparation with engine-driven nickel-titanium instruments has become popular. More recently advanced instrument designs including noncutting tips, radial lands, different cross-sections and varying tapers have been developed to improve working safety, to shorten working time and to create a greater flare within preparations (Bergmans *et al.* 2001).

To date, there has been no literature published on the apical extrusion of intracanal bacteria during root canal instrumentation. The purpose of this study was to compare *ex vivo* the number of bacteria extruded apically from extracted teeth, using two engine-driven techniques utilizing nickel-titanium instruments. ProTaper instruments (Dentsply Maillefer, Ballaigues, Switzerland) have progressively increasing tapers, a convex triangular section, and a modified guiding tip (Paqué *et al.* 2005). System GT instruments (Dentsply Maillefer) feature a U-shaped blade design, a noncutting tip, and different taper and sizes (Veltri *et al.* 2004).

Materials and methods

Selection and preparation of teeth

Forty freshly extracted human mature mandibular premolar teeth with similar dimensions were used. Digital radiographs (Schick Technologies Inc., Long Island City, NY, USA) were taken in buccal and proximal directions to check for a single canal. All teeth had similar root curvatures of 0–10°(Schneider 1971). Calcified canals and canals with large apical foramina were excluded. The teeth were cleaned of debris and soft tissue remnants and were stored in physiological saline at +4 °C until required.

Endodontic access cavities were prepared (Endo Access Bur; Dentsply Maillefer) in a high-speed handpiece. The pulp chambers were accessed, and any missing coronal teeth structure was then replaced with acid-etched composite resin (Charisma; Heraeus Kulzer, Dormagen, Germany) to create a reservoir for loading a suspension of *Enterococcus faecalis.*

Test apparatus

The schematic representation of the model system used to evaluate bacterial extrusion is presented in Fig. 1. Briefly, holes were created in the rubber stoppers of vials with a hot instrument. The tooth was inserted under pressure through the rubber stopper, which was fixed to the cementoenamel junction by means of cyanoacrylate (Quickstar; Furkan Inc., Istanbul, Turkey). Two coats of nail varnish were applied to the external surface of all roots in order to prevent bacterial microleakage through lateral canals or other discontinuities in the cementum. The rubber stopper with the tooth was then fitted into the mouth of the vial. The apical part of the root was suspended within the vial, which acted as a collecting container for apical material evacuated through the foramen of the root. The plastic part of a 23-gauge needle was removed and the needle curved and placed through the rubber stopper. Cyanoacrylate was applied at the rubber stopper/needle junction. The vial was vented with this

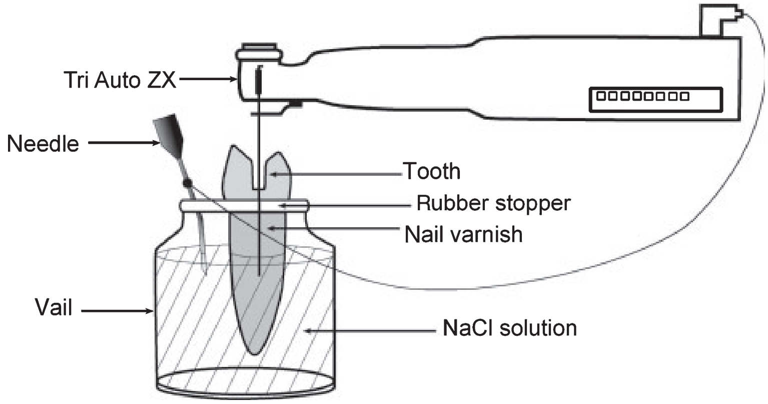

Figure 1 The schematic representation of model system.

needle during insertion to equalize the air pressure inside and outside the vial. The needle was also used as an electrode for the electronic working length determination during canal instrumentation.

The entire model system was then sterilized in ethylene oxide gas for a 12-h cycle using the Anprolene AN 74C Gas Sterilizer (Andersen Products Inc., Haw River, NC, USA).

Contamination with *E. faecalis*

A pure culture of *E. faecalis* (ATCC 29212) was used to contaminate root canals. A suspension was prepared by adding 1 mL of a pure culture of *E. faecalis*, grown in brain–heart infusion broth (Difco, Detroit, MI, USA) for 24 h, to fresh brain–heart infusion broth. Then McFarland standard number 0.5 was used to evaluate the broth to ensure that the number of bacteria was 1.5×10^8 colony-forming units (CFU) mL^{-1}. Each root canal was completely filled with the *E. faecalis* suspension using sterile pipettes. During incubation, canals were hand instrumented with a 10 K-file to carry the bacteria down the length of the canals. The contaminated root canals were then dried at 37 °C for 24 h.

Before the experiment, the vials were filled with 0.9% NaCl solution. A hole was created in the nail varnish that covered the apical foramen using a size 10 K-file. During this procedure, only 1–2 mm of instrument was extruded. In this way, a standard size of foramen and apical patency was achieved. The tooth-rubber stopper-needle unit was fitted into the mouth of the vial. The contaminated roots were divided into two experimental groups of 15 teeth each and one control group of 10 teeth.

Group 1, ProTaper group. The root canals were instrumented using ProTaper nickel-titanium instruments.

Group 2, System GT group. The root canals were instrumented using System GT nickel-titanium instruments.

Group 3, control group. No instrumentation was attempted in the teeth that served as the control group.

Before the beginning of and after the end of laboratory tests, 0.1 mL NaCl solution was taken from the experimental vials in order to count the bacteria; the suspension was incubated in brain–heart agar at 37 °C for 24 h. Colonies of bacteria were counted and the results were given as number of CFU.

Root canal preparation

One operator, using aseptic techniques, carried out the preparation and sampling procedures on each specimen under a class I laminar airflow cabinet to prevent airborne bacterial contamination. The operator was shielded from seeing the root apex during the instrumentation procedures by a rubber dam that obscured the vial.

All canal preparations and working length measurements were completed using a Tri Auto ZX (Morita, Kyoto, Japan) endodontic handpiece at low speed (300 rpm) and 'automatic reverse function' mode. The working lengths were determined as 0.5 mm shorter than the electronically detected 'apical foramen' for all the teeth.

Regardless of the technique used, all root canals were irrigated with 2 mL of 2.5% NaOCl solution between each instrument and kept flooded with irrigant during the instrumentation phase. The irrigant was delivered via an endodontic syringe with a 27-gauge blunt needle that had been placed down the canal until slight resistance was felt. At the end of instrumentation, a final irrigation was accomplished with 5 mL of 2.5% NaOCl solution.

The instrumentation sequences used in this study were as follows.

ProTaper group.
ProTaper instruments were used in a crowndown manner according to the manufacturer's instructions using a gentle in-and-out motion. Instruments were withdrawn when resistance was felt and changed for the next instrument.

A shaping file (S1) was used first and moved apically to 2 mm short of the working length. SX files were then used sequentially until resistance was encountered (4–5 mm from the working length), followed by S1 and S2 to the working length for the shaping of the coronal two-thirds of the canal. The apical one-third was finished by using F1, F2 and F3 sequentially to the working length, with only one pecking motion for each instrument.

Once the instrument had negotiated to the end of the canal and had rotated freely, it was removed.

System GT group.
System GT instruments were also used in a crowndown manner according to the manufacturer's instructions using a gentle in-and-out motion. Instruments were withdrawn when resistance was felt and changed for the next instrument.

Instruments of size 20, .12 taper, 20, .10 taper, 20, .08 taper and 20, .06 taper were used sequentially until progression became difficult. For coronal flaring, a size 20, .12 taper instrument was used to a depth of 6 mm, a size 20, .10–4 mm, a size 20, .08–2 mm from the working length and a size 20, .06 to the working length. Then size 25, .04 and size 30, .04 instruments were used for apical shaping.

Once the instrument had negotiated to the end of the canal and had rotated freely, it was removed.

Control group.
After contamination and apical perforation, 10 teeth were choosen and maintained in the test medium. Subsequently, 0.1 mL NaCl was taken from the experimental vials for counting the bacteria and incubated in brain–heart agar. Colonies of bacteria were counted and the results were given as CFU.

Statistical analysis

Statistical tests were performed using SPSS (Version 9.0; SPSS Inc., Chicago, IL, USA). Data were analysed statistically using Kruskal–Wallis one-way analysis of variance and Mann–Whitney *U*-tests. The level of statistical significance was set at $P = 0.05$.

Results

No growth was observed when checking the sterility of the whole apparatus. The mean number of extruded bacteria for the groups are presented in Table 1.

Comparison of the mean number of extruded bacteria between ProTaper-control and System GT-control groups showed statistically significant differences ($P < 0.05$). However, the difference between ProTaper and System GT groups was not statistically significant ($P > 0.05$).

Discussion

The aim of this study was to assess the apical extrusion of intracanal bacteria as a result of root canal shaping by two different engine-driven nickel-titanium instruments. Common to all techniques were the amount and type of irrigant and the operator. To increase the probability that the amount of apically extruded bacteria was a result of instrumentation, a standardized tooth model was used to decrease the number of variables. The teeth used for this study were carefully selected according to tooth type, canal size at the working length and canal curvature. This ensured that the number of apically extruded bacteria was due to the instrumentation technique and not to tooth morphology.

In this study, working length measurements were completed with a Tri Auto ZX electronic apex locating handpiece with 'autoreverse function mode'. During the experiment, the lip clip was connected to needle and NaCl solution was used as a conducting medium. The working lengths were determined 0.5 mm short of the apical foramen for all the teeth. Also, the size of the master apical instrument was kept constant; the tip diameter of a ProTaper F3 instrument and a ProFile size 30 (.04 taper) are normally the same as a size 30 K-Flexofile (0.3 mm at D_0).

Table 1 The mean number of extruded bacteria

Groups	Total (*n*)	Mean (CFU mL^{-1})	SD
ProTaper	15	6.9	3.3
System GT	15	7.8	3.6
Control	10	0.5	0.2

KW = 15.86; $P < 0.05$; SD, standard deviation.

It is well-documented in the literature that contaminated as well as noncontaminated intracanal materials can trigger an inflammatory reaction when forced apically during root canal preparation. Seltzer *et al.* (1968) reported that even sterile dentine debris in the periapical area was associated with persistent inflammation. Torneck *et al.* (1973) reported similar findings in the incisors of young primates. When root canal treatment is performed in contaminated canals, an analogous situation may exist in a patient with a chronic pulpitis or pulp necrosis, especially when an apical periodontitis exists. Seltzer & Naidorf (1985) reported that new irritants in the form of chemically altered pulp tissue proteins may be introduced into the granulomatous lesion and that a violent reaction may follow. Naidorf (1985) demonstrated the presence of immunoglobulins in the periapical areas. He also showed that some of the immunoglobulins are related to the antigens in the canals. It is easy to understand that if the canal contains antigens and a granuloma has antibodies, when intracanal contents are pushed through, it will result in an antigen–antibody complexing. This reaction will cause damage to the cell membrane resulting in prostaglandin release, bone resorption, amplification of the kinin system and ultimately pain for the patient (Ruiz-Hubard *et al.* 1987). Furthermore, Mathiesen (1973) and Perrini & Fonzi (1985) have found numerous mast cells in human periapical lesions. Based on this information Torabinejad *et al.* (1985) concluded that physical or chemical injury of periradicular tissues during root canal preparation can cause degranulation of mast cells in periapical tissues. Mast cells discharging vasoactive amines into the periapical tissues, initiate an inflammatory response or aggravate an existing inflammatory process. Also, Kayaoglu & Ørstavik (2004) reported that some bacterial species resistant to killing by the elements of the host defence have the potential to sustain inflammatory response and to delay healing when translocated from the root canal into the periapical lesion. Aside from local effects, extrusion of microbes into periradicular tissues during endodontic treatment has the potential to bring about serious systemic disease such as endocarditis, brain abscesses and septicaemia, particularly in compromised patients (Debelian *et al.* 1994, Savarrio *et al.* 2005). Therefore, every effort should be exerted to limit the periapical extrusion of intracanal materials during treatment.

The extrusion produced by the various techniques was expected, because it is considered a problem of all canal instrumentation techniques (Vande Visse & Brilliant 1975). The results of this study demonstrated that the instrumentation techniques tested (ProTaper and System GT) created apically extruded bacteria *ex vivo*. However, there was no statistical difference between the two engine-driven techniques in terms of extrusion of microorganism.

In this study, a crowndown technique was used for such engine-driven systems. As the greatest number of microorganisms in the root canal lie in the coronal third (Shovelton 1964) initial preparation of this section of the root canal system helps to reduce the number of microorganisms that may be pushed apically. Secondly, early flaring of the coronal part of the preparation may improve instrument control during preparation of the apical third of the canal (Goerig *et al.* 1982).

Many factors affect the amount of extruded intracanal materials such as; instrumentation technique, instrument type, instrument size and preparation endpoint and irrigation solution (Vande Visse & Brilliant 1975, Salzgeber & Brilliant 1977, Fairbourn *et al.* 1987, Al-Omari & Dummer 1995, Beeson *et al.* 1998, Hinrichs *et al.* 1998, Reddy & Hicks 1998, Ferraz *et al.* 2001, Azar & Ebrahimi 2005, Tinaz *et al.* 2005).

Enterococcus faecalis was chosen as the bacteriological marker in this study. It is a nonfastidious, easy-to-grow aerobic bacterium of significant clinical importance, that could be used in a study applying a bacteriological assessment method. Other bacteria commonly associated with endodontic infections may require symbiotic support from other bacteria, but *E. faecalis* has been reported to survive and successfully thrive alone (Dahlen & Haapasalo 1998, Portenier *et al.* 2003).

Conclusion

Overall, both engine-driven nickel-titanium techniques were extruded intracanal bacteria through the apical foramen. However, no significant difference was found in number of CFU between ProTaper and System GT.

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